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MicroRNA-100 regulates osteogenic differentiation of human adipose-derived mesenchymal stem cells by targeting BMPR2

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ABSTRACT

Elucidation of the molecular mechanisms governing human adipose-derived mesenchymal stem cells (hASCs) osteogenic differentiation is of great importance for improving the treatment of bone-related diseases. In this study, we examined the role of microRNA (miR)-100 on the osteogenesis of hASCs. Overexpression of miR-100 inhibited osteogenic differentiation of hASCs in vitro, whereas downregulation of miR-100 enhanced the process. Target prediction analysis and dual luciferase report assay confirmed that bone morphogenetic protein receptor type II (BMPR2) was a direct target of miR-100. Furthermore, knockdown of BMPR2 by RNA interference inhibited osteogenic differentiation of hASCs, similar as the effect of upregulation miR-100. Taken together, our findings imply that miR-100 plays a negative role in osteogenic differentiation and might act through targeting BMPR2.
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1. Introduction

Human adipose-derived mesenchymal stem cells (hASCs) have been reported to be a population of self-renewing and multipotent cells that might have clinical therapeutic potentials [1,2]. They can differentiate into several lineages including osteoblasts in response to stimulation by multiple environmental factors [3]. However, the regulation of the molecular pathways is not fully elucidated. Investigation the molecular mechanism of osteogenic differentiation of hASCs could provide a better understanding of the pathogenesis of skeletal diseases such as osteoporosis and may lead to the development of new strategies for therapies.

MicroRNAs (miRNAs) are endogenous small (19–25 nt) non-coding RNAs that regulate their targets by incomplete complementation to nucleotides within the 3′ untranslated region (3′-UTR) or open reading frame (ORF) of coding mRNAs, resulting in suppressing gene expression by inhibiting translation, promoting mRNA decay or both [4]. They have emerged as key regulators of various biological and pathological processes [5]. The effect of miRNAs has also been investigated in the osteogenesis of hASCs. For instance, miR-26a has been reported to repress the translation of the osteogenic marker SMAD1 and inhibit the osteogenesis of hASCs [6]. Recently, the same research group found an upstream regulator of miR-26a, menin, silencing of which resulted in downregulation of miR-26a, with a consequent upregulation of SMAD1 protein, suggesting a novel target for bone disease RNA-based therapy [7]. Besides, overexpression of miR-196a regulates HOXC8, enhances osteogenic differentiation, and decreases hASCs proliferation [8], whereas overexpression of miR-146a induced the inhibition of IRAK1 expression and inhibited osteogenic differentiation of hASCs [9]. Moreover, we found that miR-22 could keep the balance of hASCs osteogenic and adipogenic differentiation, upregulation of which could promote osteogenic differentiation but inhibit adipogenic differentiation by repressing HDAC6 protein expression [10]. Meanwhile, the impact of miRNAs on osteogenic differentiation of other cell types has also been examined by modulation of miRNA function [11], showing the significant importance of miRNA regulation on this biological process.

In this study, we demonstrated the inhibitory function of miR-100 in osteogenic differentiation of hASCs. The expression profiles of miRNAs during osteogenic differentiation of hASCs were obtained by miRNA microarray analysis and miR-100 was found to be significantly downregulated. We analyzed the function of miR-100 in

Abbreviations: hASCs, human adipose-derived mesenchymal stem cells; miRNA, microRNA; 3′UTR, 3′untranslated region; ORF, open reading frame; mRNAs, messenger RNAs; qRT-PCR, quantitative reverse transcription-polymerase chain reaction; BMP, bone morphogenetic protein; BMPR2, bone morphogenetic protein receptor type II; RUNX2, runt-related transcription factor 2; ALP, alkaline phosphatase; OC, osteocalcin; OPN, osteopontin

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Table 1
Primers used in this study.

Name	Forward (5′–3′)	Reverse (5′–3′)
RUNX2	TGTCATGGCGGGTAACGAT	AAGACGGTTATGGTCAAGGTGAA
ALP	CCACGTCTTCACATTTGGTG	AGACTGCGCCTGGTAGTTGT
OC	GGCGCTACCTGTATCAATGG	GTGGTCAGCCAACTCGTCA
OPN	ACTCGAACGACTCTGATGATGT	GTCAGGTCTGCGAAACTTCTTA
BMPR2	CACTCAGTCCACCTCATTCATTT	TTGTTTACGGTCTCCTGTCAAC
GAPDH	GGTCACCAGGGCTGCTTTTA	GGATCTCGCTCCTGGAAGATG

osteogenic differentiation by transfection of exogenous miR-100 mimics or its inhibitor and validated the regulatory relationship between miR-100 and its target BMPR2.

2. Materials and methods

2.1. hASCs isolation and culture

hASCs were isolated from adipose tissues which obtained from patients undergoing tumescent liposuction according to procedures approved by the Ethics Committee at the Chinese Academy of Medical Sciences and Peking Union Medical College. hASCs were then resuspended at a density of 2×10^6 in 12 mL regular growth medium [12] and cultured in a 75 cm^2 flask after isolation from human adipose tissue. Cell cultures were maintained at 37 °C in a humidified incubator with 5% CO_2. Passage 3 cells were used for following experiments.

2.2. Osteogenic differentiation in vitro

hASCs were plated at a cell density of 2×10^5 cells in 6-well plates. At 80% confluence, medium was replaced with osteoblasts-specific induction medium containing high glucose of Dulbecco's Modified Eagle's Medium (H-DMEM), 10% FBS, 10 mmol/L β-glycerophosphate, 1×10^{-9} mol/L dexamethasone, and 0.2 mM antiscorbic acid (all purchased from Sigma–Aldrich) to induce the differentiation. HASCs were cultured in induction medium for 15 days. The induction medium was changed every 3 days.

2.3. miRNA and siRNA transfection

The synthetic miR-100 mimics, miR-100 inhibitor, negative control of mimics and negative control of inhibitor were purchased from GenePharma (GenePharma, Inc., Shanghai, China). The synthetics were transfected into hASCs at the final concentration of 200 nM using lipofectamine 2000 (Invitrogen, USA), as per the manufacturer's instructions. The whole transfection process was proceeded in a non-serum medium named opti-mem (Gibco, USA) for 6 h at 37 °C in a humidified environment containing 5% CO_2. After transfection, the medium was changed into induction medium for differentiation or into regular growth medium for other experiments such as fluorescence detection. Three pairs of siRNA of BMPR2 were designed and synthesized (GenePharma, Inc., Shanghai, China) and they were transfected into hASCs with the same procedure as miRNA transfection.

2.4. Alkaline phosphatase staining and Alizarin Red staining

The osteoblast phenotype was evaluated by determining alkaline phosphatase (ALP) activity. ALP staining was normally performed at days 3, 6 and 9 using ALP staining kit (Blood institute, Chinese Academy of Medical Sciences) followed by the accessory procedure. Alizarin Red staining was performed to detect the calcification at late period of induction. Cells in 24-well plates were washed with PBS,

Table 2
Constructed sequences used in this study.

Name	Forward (5′–3′)	Reverse (5′–3′)
P-BMPR2-WT	ctagaAGAAAATACTCGCACTTCCTCAGAACCCTCTTTCTTGTTA*ACGGGTA*TCTTTTG TTGGTGTGTTTTGCTCTTACATTACAGATAGACgc	ggccgcGTCTATCTGTAATGTAAGAGCAAAACACACCAACAAAAGA *TACCCGT*TAACAAGAAAGAGGGTTCTGAGGAAGTGCGAGTATTTTCTt
P-BMPR2-MUT	ctagaAGAAAATACTCGCACTTCCTCAGAACCCTCTTTCTTGTTA*CTTAAGC*TCTTTTGTTG GTGTGTTTTGCTCTTACATTACAATAGACgc	ggccgcGTCTATCTGTAATGTAAGAGCAAAACACACCAACAAAAGA *GCTTAAG*TAACAAGAAAGAGGGTTCTGAGGAAGTGCGAGTATTTTCTt

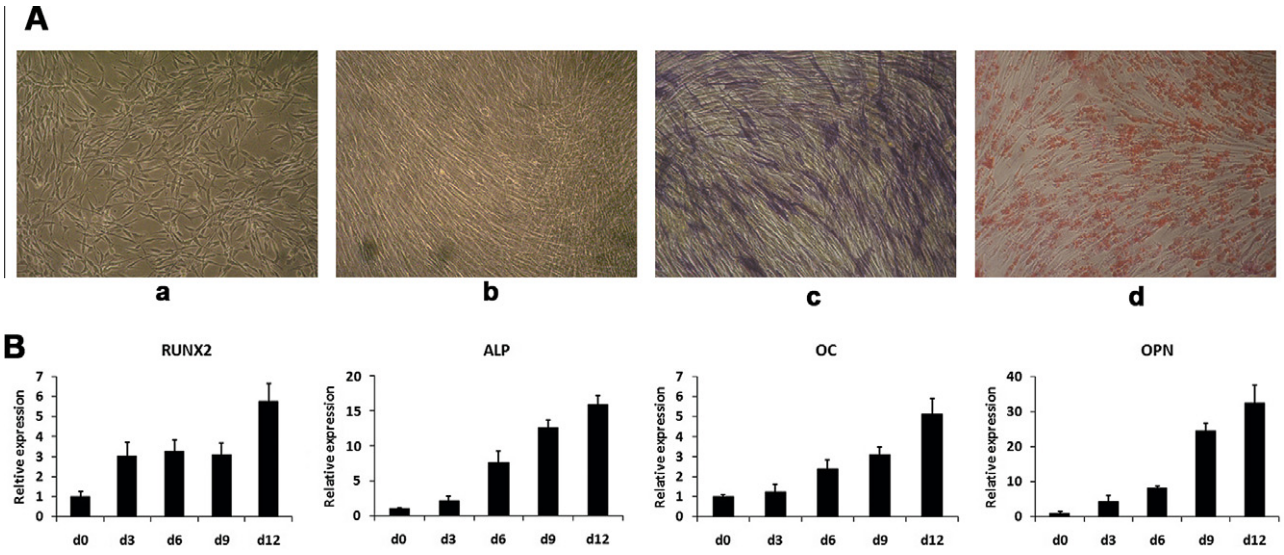

Fig. 1. The morphology of hASCs and their osteogenic differentiation. (A) The morphology of hASCs (a) and osteoblasts induced from hASCs (b); ALP staining (c) at day 6 and Alizarin Red staining (d) at day 15 of osteoblasts induced from hASCs (Magnification, 40×). (B) qRT-PCR analysis showed the increasing osteogenic specific genes runt-related transcription factor 2 (*RUNX2*), alkaline phosphatase (*ALP*), osteocalcin (*OC*), and osteopontin (OPN). The data, normalized to GAPDH are averages of three independent experiments (mean ± SD).

fixed in 95% ethanol for 10 min, washed with distilled water, and stained by Alizarin Red solution (1 g Tris and 0.1 g Alizarin Red (Sigma–Aldrich, USA) in 100 mL ultrapure water; adjusting the pH to 8.3 by HCl) for 30 min at 37 °C. After washing twice with water, the cells were photographed.

2.5. Quantitative real-time polymerase chain reaction

Cultured cells were lysed by TRIzol (Invitrogen, USA) and RNA isolation and reverse transcription were performed as previously described [13]. Real Time PCR amplification was performed in triplicates according to procedures reported previously [13]. Relative

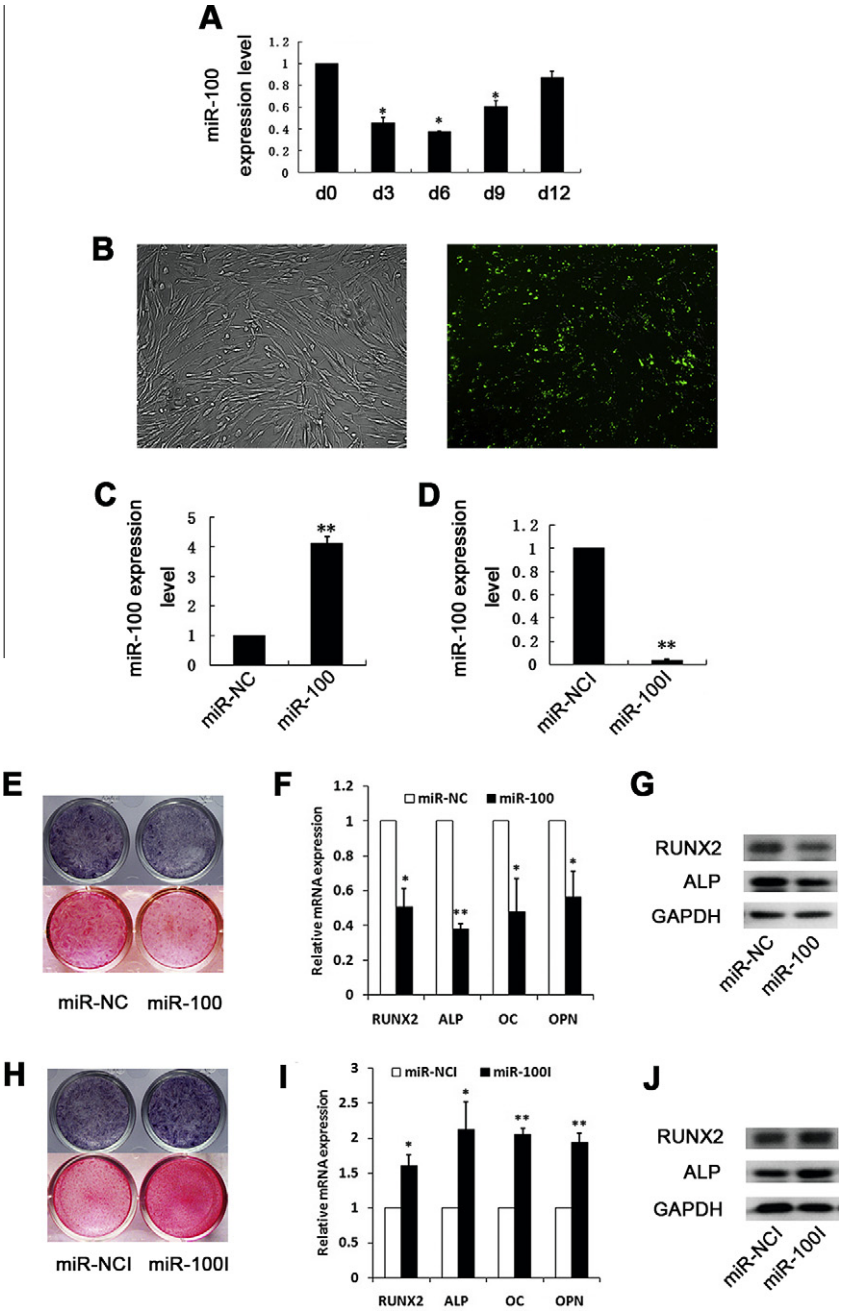

Fig. 2. Overexpression of miR-100 inhibits osteogenic differentiation of hASCs while inhibition of miR-100 promotes the process. (A) The expression of miR-100 during osteogenesis of hASCs was quantitatively assessed by quantitative real-time polymerase chain reaction (qRT-PCR) at the indicated time points. The data are normalized to U6. (B) The efficiency of microRNA (miRNA) transfection was estimated to be 80–90% (Magnification, 40×). (C) The expression of miR-100 in hASCs transfected with miRNA mimics was determined by qRT-PCR. $^{**}P < 0.01$ compared with negative control (miR-NC)-transfected hASCs. (D) The expression of miR-100 in hASCs transfected with miRNA inhibitor (miR-100I) was determined by qRT-PCR. $^{**}P < 0.01$ compared with negative control of inhibitor (miR-NCI)-transfected hASCs. (E, H) ALP staining (upper) at day 6 and Alizarin Red staining (lower) at day 15 showed the inhibited or enhanced ALP activity and calcification of osteogenic differentiation after transfection with miR-100 mimics or miR-100 inhibitor compared with their separate negative control. (F, I) The expression of osteogenic specific genes *RUNX2*, *ALP*, *OC*, and *OPN* after transfection with miR-100 mimics or miR-100 inhibitor was analyzed by qRT-PCR. The data are normalized to *GAPDH*. $^{*}P < 0.05$ or $^{**}P < 0.01$ compared with separate negative control. (G, J) Western blotting results showed the accordant protein expression of RUNX2 and ALP with the mRNA expression. All the data represent mean ± SD of three independent experiments, otherwise, one of three independent experiments is shown. miR-NC refers to negative control of miRNA mimics; miR-100 refers to miR-100 mimics; miR-NCI refers to negative control of miRNA inhibitor or anti-miR; miR-100I refers to miR-100 inhibitor.

expression of mRNA or microRNA was evaluated by $2^{-\Delta\Delta Ct}$ method and normalized to the expression of GAPDH or U6 respectively. The primer of the related genes lists in Table 1.

2.6. Western blotting analysis

After washing twice with PBS, cells were lysed in ice-cold Radio Immunoprecipitation Assay (RIPA) lysis buffer (Beyotime, Nanjing, China) and manually scraped from culture plates. Proteins were separated on 10% sodium dodecyl sulphate–polyacrylamide gel electrophoresis gels, electroblotted onto a polyvinylidene difluoride membrane, and blotted with the primary anti-BMPR2 antibody (1/1000; Cell Signaling Technology, USA), anti-RNUX2 antibody (1/200; Santa Cruz Biotechnology, USA), anti-ALP antibody (1/100; Santa Cruz Biotechnology, USA) or anti-GAPDH antibody (1/2000; Santa Cruz Biotechnology, USA) and secondary anti-rabbit or anti-mouse horseradish peroxidase-conjugated antibody (Zhongshan, Beijing, China). Antibody and antigen complexes were detected using chemiluminescent ECL reagent (Millipore, USA).

2.7. Dual luciferase reporter genes construct

A 94 bp fragment of the BMPR2 3′UTR containing the predicted binding site 40 bp flanking sequence on each side for hsa-miR-100 and its mutant fragment was synthetized by company (Sangon, Shanghai, China) with a short extension containing cleavage sites (Table 2) for XbaI (5′ end) and NotI (3′ end). The constructed plasmids were termed as P-BMPR2-WT (BMPR2-wild type) and P-BMPR2-MUT (BMPR2-mutation), respectively. Amplicons were cleaved with XbaI and NotI and cloned in between the XhoI and NotI cleavage sites of the pRL-TK vector (Promega Corporation, USA) downstream of the Renilla luciferase reporter gene.

2.8. Dual luciferase reporter assay

The sequence of miR-100 binding site and mutant site were shown in italics in Table 2. Each vector, along with 100 ng pGL3 and 200 nmol/L miR-100 mimics or miR-NC, was transfected into 293T cells using Lipofectamine 2000 reagent (Invitrogen, USA) as previously reported [12]. Cells were harvested 24 h after transfection and assayed for renilla and firefly luciferase activity using the Dual-Luciferase Reporter Assay System (Promega Corporation, USA).

2.9. Statistical analysis

Data are presented as mean ± SD. Comparisons between groups were analyzed via Paired-Samples *T* Test using SPSS 17.0. Statistical significance was defined as *P* value <0.05.

3. Results

3.1. hASCs osteogenic differentiation

hASCs (Fig. 1A. a) could be induced into osteoblasts (Fig. 1A. b), and the osteoblasts were evidenced by ALP staining (Fig. 1A. c) and Alizarin Red staining for matrix mineralization (Fig. 1A. d). qRT-PCR analysis showed increased expression of osteogenic specific genes, runt-related transcription factor 2 (*RUNX2*), *ALP*, osteocalcin (*OC*), and osteopontin (*OPN*), at days 3, 6, 9, and 12 after induction (Fig. 1B).

3.2. MiR-100 inhibits osteogenesis of hASCs

hASCs were harvested at different time points during osteogenic differentiation and the expression of miR-100 was determined by qRT-PCR. As shown in Fig. 2A, the expression of miR-100 decreased by more than 50% at day 3 compared with that of untreated control hASCs (day 0) and maintained at a low level until day 9, suggesting that miR-100 might negatively regulate osteogenic differentiation.

To determine the role of miR-100 in osteogenic differentiation, hASCs were transfected with miR-100 mimics or inhibitor and respective negative control at day 0 and day 3 to reinforce the effect of miRNAs; and then the osteogenesis ability was examined. The efficiency of transfection was estimated to be 80–90% and similar among the groups according to the observation of uptake of a fluorescent small RNA duplex oligonucleotide by a fluorescent microscope (Fig. 2B). According to qRT-PCR analysis, a fourfold increase of mature miR-100 expression was shown in hASCs 24 h post transfection of miR-100 mimics (Fig. 2C), and a 25-fold decrease after transfection of miR-100 inhibitor (Fig. 2D). The osteogenic induction was conducted at d0 after transfection. At day 6 of differentiation, ALP staining (Fig. 2E upper) showed a significant lessened difference in miR-100 group compared with miR-NC group, whereas, after inhibition of miR-100, the outcome was totally inversed (Fig. 2H upper). The Alizarin Red staining (Fig. 2E

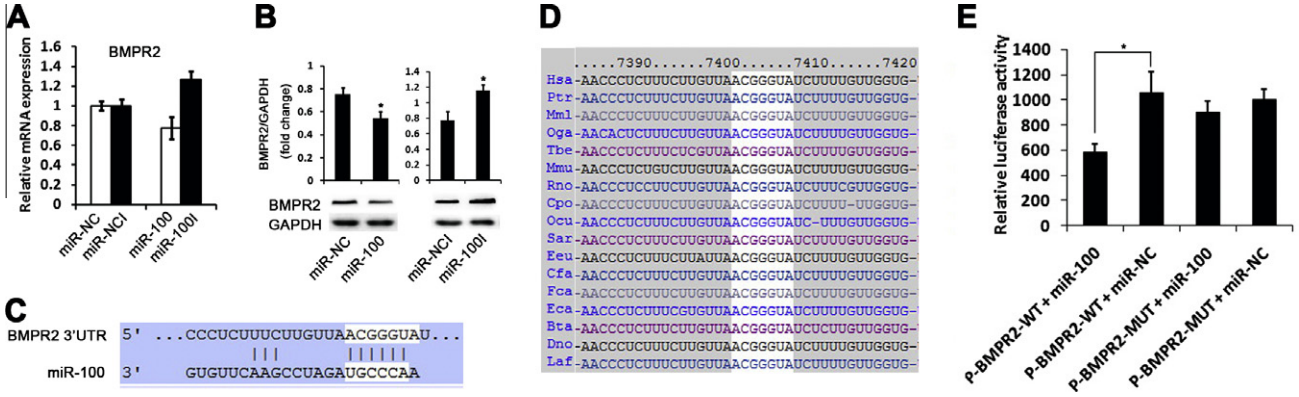

Fig. 3. miR-100 targets the 3′ untranslated region (3′-UTR) of BMPR2 mRNA. (A) hASCs were transfected with miR-100 mimics (inhibitor) or miR-NC (NCI), and then the mRNA expression of BMPR2 was analyzed by qRT-PCR. Data represent mean ± SD of three independent experiments. (B) One of three independent Western blotting analyses showed the BMPR2 protein expression, and the quantitative determination represents mean ± SD of three independent experiments. $^{*}P < 0.05$. (C) miR-100 is partially complementary to a region in the BMPR2 3′-UTR. The complementary miR-100-binding site found in the BMPR2 3′-UTR or mutant site was inserted into downstream of the luciferase reporter plasmid pRL-TK. (D) The predicted, single binding site of miR-100 (unshaded) in the 30-UTR of BMPR2 is conserved among vertebrates. (E) P-BMPR2-WT or P-BMPR2-MUT luciferase constructs were simultaneously transfected into cells with plasmid pGL-3 and miR-100 mimics or miR-NC. Renilla luciferase activity was normalized to firefly luciferase which was conducted by pGL-3. Data represent mean ± SD of three independent experiments. $^{*}P < 0.05$ compared with cells transfected with miR-NC and P-BMPR2-WT.

lower and Fig. 2H lower) at day 15 showed the same tendency at the matrix mineralization level. Key osteogenic-specific genes were significantly downregulated at day 6 of differentiation due to the overexpression of miR-100 (Fig. 2F) but upregulated after inhibition of miR-100 (Fig. 2I). We also examined the RUNX2 and ALP protein expression (Fig. 2G and J), which were compatible with the qRT-PCR results. Therefore, overexpression of miR-100 resulted in osteogenesis inhibition of hASCs but downregulation of miR-100 enhanced the osteogenesis.

3.3. BMPR2 is a direct target of miR-100

The conserved target genes of miR-100 were searched and predicted by TargetScan and microrna.org to better understand the molecular mechanisms that underlie miR-100-mediated regulation of osteogenic differentiation. Among the predicted candidates, we identified bone morphogenetic protein type II receptor (BMPR2), a kinase receptor of BMPs with an established role in osteogenesis [14–16]. To determine whether miR-100 could regulate the expression of BMPR2, mRNA and protein of BMPR2 were examined in miR-100 overexpressed or downregulated hASCs. As shown in Fig. 3A, there's a slight change in BMPR2 mRNA level, however, BMPR2 protein in hASCs that overexpressed miR-100 was obviously lower than that in control cells 48 h post transfection but downregulation of miR-100 could increase the BMPR2 protein expression (Fig. 3B). According to the analysis of TargetScan and microran.org, miR-100 targeting site in the 3′-UTR of *BMPR2* is highly conserved among vertebrates and partially complementary to miR-100 (Fig. 3C and D). These data indicated that the translation of BMPR2 protein might be regulated by miR-100. To test this hypothesis, a standard luciferase reporter assay was conducted in 293T cells. After cotransfection with luciferase construct, internal control vector pGL3 and miRNA, the cells were harvested at 48 h and analyzed for dual luciferase activity. Results

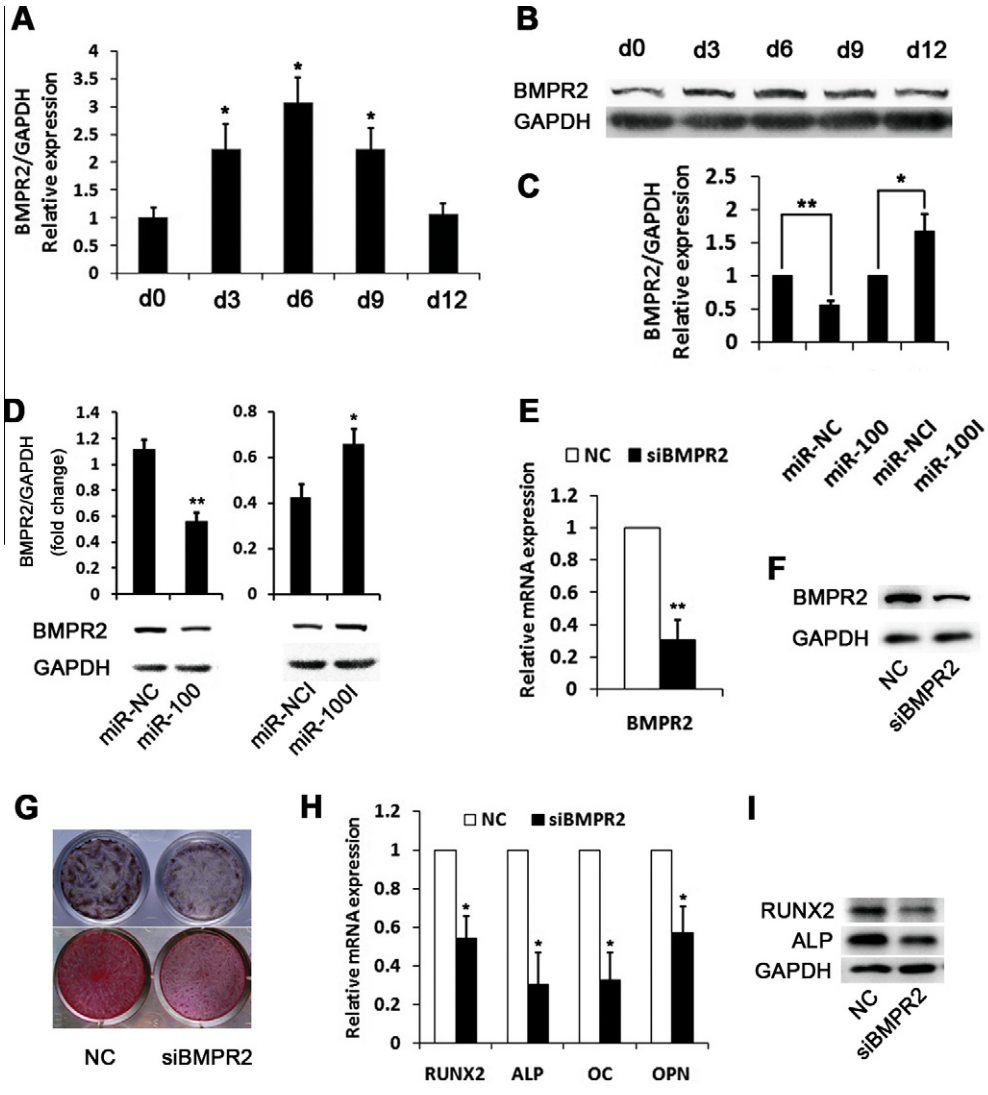

Fig. 4. Knockdown of BMPR2 by RNA interference inhibits osteogenic differentiation of hASCs. (A) BMPR2 mRNA expression during osteogenesis of hASCs was analyzed by qRT-PCR. $^{*}P < 0.05$, as compared with day 0. (B) BMPR2 protein expression during osteogenesis of hASCs was analyzed by western blotting. (C) mRNA expression of *BMPR2* at day 6 was compared between miR-NC and miR-100 transfection group, or miR-NCI and miR-100I group. $^{*}P < 0.05$, $^{**}P < 0.01$. (D) BMPR2 protein expression at day 6 was detected by western blotting, and also showed by quantitative determination. $^{*}P < 0.05$ and $^{**}P < 0.01$. (E, F) After siRNA interference of BMPR2 in hASCs, qRT-PCR and western blotting analysis were used to test the expression of BMPR2. $^{**}P < 0.01$. (G) ALP staining (upper) at day 6 and Alizarin Red staining (lower) at day 15 showed the inhibited ALP activity and calcification of osteogenic differentiation after BMPR2 knockdown. (H) qRT-PCR analysis of the expression of the osteogenic specific genes *RUNX2*, *ALP*, *OC*, and *OPN* at day 6 after siRNA interference. The data are normalized to *GAPDH*. $^{*}P < 0.05$ compared with NC group. (I) Western blotting results showed the accordant protein expression of RUNX2 and ALP with the mRNA expression. All the data represent mean ± SD of three independent experiments, otherwise, one of three independent experiments is shown.

showed that the renilla luciferase activity for P-BMPR2-WT-transfected cells decreased by more than 40% in miR-100 mimics-cotransfected cells compared with that in miR-NC-cotransfected cells. In addition, site-directed mutagenesis of the seed region abolished the inhibitory effect of miR-100 mimics (Fig. 3E). Taken together, these results revealed that miR-100 could regulate BMPR2 protein expression through a partially complementary binding site in the 3′-UTR of BMPR2.

3.4. BMPR2 downregulation inhibits osteogenic differentiation

Next, we studied the expression pattern of BMPR2 during osteogenic differentiation. qRT-PCR and western blotting analysis revealed that expression of BMPR2 was increased during osteogenic differentiation of hASCs (Fig. 4A and B) and it reached the peak at day 6, while the expression level of miR-100 was at the lowest at day 6 (Fig. 2A). These data implied a possible corelationship between BMPR2 and miR-100 when hASCs were being induced into osteoblasts. Additionally, qRT-PCR and western blotting results showed that BMPR2 was downregulated after transfection of miR-100 mimics in the process of osteogenesis, but upregulated after transfection of miR-100 inhibitor (Fig. 4C and D). We carried out mRNA knockdown to check if BMPR2 plays a positive role in the osteogenesis process. qRT-PCR analysis illustrated that endogenous BMPR2 mRNA expression was reduced by three times (Fig. 4E) 48 h after transfected with siBMPR2 compared with negative control (NC), which was also accordant with western blotting results (Fig. 4F). Osteogenesis induction was conducted while hASCs were transfected with siBMPR2 or NC at day 0 and d3, same time point with transfection of miRNAs. The osteogenic differentiation was inhibited as demonstrated by a lack of ALP activity and calcification (Fig. 4G), similar to the effect of miR-100 overexpression. Knockdown of BMPR2 also caused the downregulation of key osteogenic-specific genes expression in the osteogenic differentiation of hASCs (Fig. 4H and I).

3.5. BMPR2 knockdown could block the effect of miR-100

To further investigate the relationship between miR-100 and BMPR2, we transfected miR-100 inhibitor into hASCs after BMPR2 knockdown and then performed osteogenic differentiation. As shown from the ALP and Alizarin Red staining (Fig. 5A), qRT-PCR analysis (Fig. 5B) and western blotting results (Fig. 5C), adoption of miR-100 inhibitor after transfection of NC could still improve the osteogenic differentiation, but inhibition of miR-100 could no longer enhance the osteogenesis of hASCs after BMPR2 knockdown. From a sidewise approach, we demonstrated that deletion of the target could block the effect the miR-100 inhibition, further indicating that miR-100 regulated the osteogenesis of hASCs through BMPR2.

4. Discussion

A further appreciation of the osteogenesis of stem cells could lead to more efficient manipulation of the pathogenesis of skeletal diseases and develop possible regenerative therapies [17]. Recently, dozens of miRNAs are emerging as important negative or positive regulators of posttranscriptional gene expression and are considered critical for osteogenesis. In this study, we identified miR-100 as a negative regulator of hASCs osteogenic differentiation. Our data revealed that miR-100 was downregulated during osteogenic differentiation of hASCs. Overexpression of miR-100 inhibited osteogenic differentiation, whereas inhibition of miR-100 function enhanced the osteogenic potential.

It has been reported that miR-100 was aberrantly expressed in some tumor samples [18–20] and served as tumor suppress regulator by targeting different genes. Here we found out a new role of miR-100 in the osteogenesis of hASCs. To study the molecular mechanism by which miR-100 regulates osteogenic differentiation of hASCs, a search with Targetscan and microrna.org revealed that

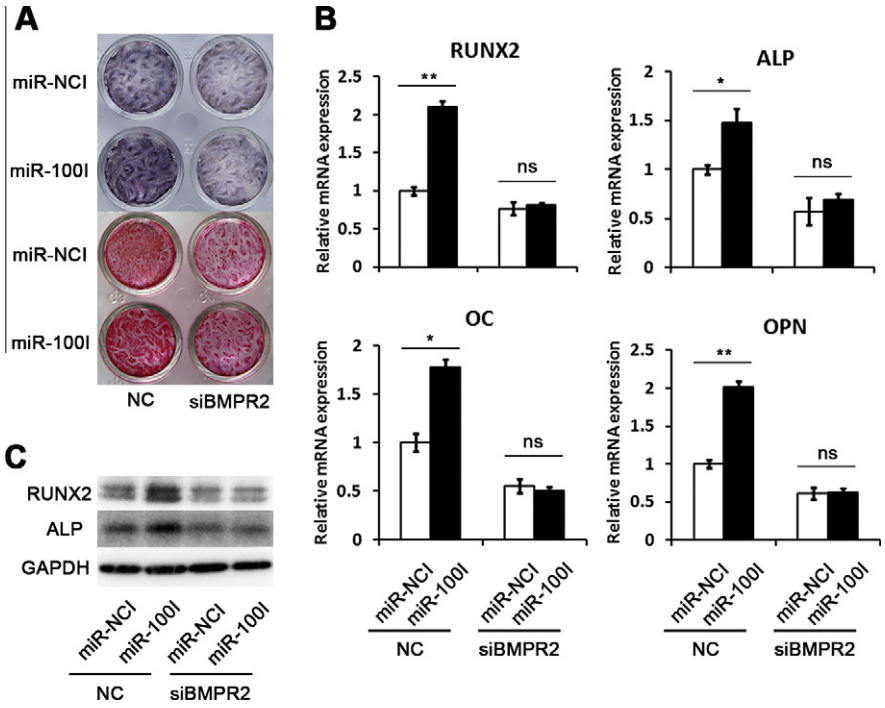

Fig. 5. BMPR2 knockdown block the effect of miR-100. (A) miR-100I and miR-NCI was transfected into the cells after siBMPR2 and NC transfection, four groups were stained to show ALP expression at day 6 (upper) and calcification at day 15 (lower). (B) The expression of osteogenic specific genes was analyzed by qRT-PCR among four groups. The data are normalized to *GAPDH*. (C) Western blotting results showed the accordant protein expression of RUNX2 and ALP. All the data represent mean ± SD of three independent experiments, otherwise, one of three independent experiments is shown.

BMPR2 might be a possible target with 9nt inconsecutive match site complementary to miR-100 in the 3′UTR of it. We showed that miR-100 overexpression resulted in downregulation of BMPR2 at the protein level, whereas functional inhibition of miR-100 led to derepression of BMPR2, strongly suggesting that BMPR2 is regulated by miR-100 during osteogenic differentiation. Meanwhile, dual luciferase reporter assay identified BMPR2 as a direct target of miR-100.

Bone morphogenetic protein (BMP) signaling have been reported to play a very important role in the commitment of hASCs osteogenic differentiation [21] and BMPs have important clinical significance serving as strong osteoinductive factors for bone tissue repair and regeneration [22]. BMP signaling is initiated by the binding of extracellular BMP to heterodimeric BMP receptors (BMPR), resulting in BMP type II receptor (BMPR2)-mediated activation of the BMPR type I receptor (BMPR1), which, in turn, causes the phosphorylation and activation of intracellular Smad signaling molecules, and then activates osteoblast-essential genes [14–16]. These reports indicated that BMPR2 might be a crucial factor in the pathway of osteogenic differentiation.

Our results showed that BMPR2 increased in the process of osteogenesis of hASCs in contrast to the expression of miR-100. We carried out BMPR2 knockdown and detected obvious osteogenic inhibition, supposing that BMPR2 did play important role in the osteogenesis of hASCs. Besides, deletion of BMPR2 could block the effect of miR-100 inhibition, indirectly illustrating the regulatory relationship between miR-100 and BMPR2. Furthermore, the expression of RUNX2 which was a downstream gene of BMP signaling [23], significantly reduced after transfection of miR-100 mimics or siBMPR2 but increased in the absence of miR-100 supports the notion that inhibition of osteoblast differentiation by miR-100 was caused at least partially by suppression of the BMPR2 downstream pathway.

In summary, miR-100 was identified as a novel regulator in human osteogenesis through its target gene BMPR2 in hASCs. BMPR2 and miR-100 might be potential therapeutic targets in the management of skeletal diseases.

Acknowledgments

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